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Extraction of crude extract from *Malva neglecta* leaves and evaluation of its free radical scavenging activities

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Introduction: Accumulation of free radicals could lead to permanent oxidative destruction of organisms by attacking macromolecules and organelles of the body, thus causing organism aging, fatigue and degenerative diseases. Therefore, discovering and developing safe and non-toxic natural antioxidants has been an interesting topic in recent decades. Compared to synthetic antioxidants, extracts from plant resources usually have quite stable constructions and great antioxidant activities, and can easily be absorbed without dangerous immunoreactions. Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radicals or active oxygen scavengers. *Malva neglecta* (MN), which is known as Panirak/Tooleh in Iran is an annual and herbaceous plant. The leaves and flowers of MN are used in traditional phytotheraphy and in treatment of cough, respiratory system and digestive system problems. The objective of this study was to optimize process conditions of extraction in order to obtain the highest yield from crude extract of MN leaves and identify its antioxidant properties.

Materials and methods: Three-variable-three-level Box-Behnken design-response surface methodology (BBD-RSM) with five replications at central point was used to optimize the extracting parameters of crude extract from the MN leaves including extraction time (1-8 h), extraction temperature (50-100°C), and the water/solid ratio (3-30). The ability of extracted materials to scavenge the free radicals of DPPH, OH and super oxide was also evaluated by chemical analysis.

Results and Discussion: All three independent variables effected the yield of crude extract of MN leaves. The extraction yield of MN crude extract significantly increased from 5.90% to 8.01% with extraction time varying from 1 h to 6 h. However, the yield was not altered meaningfully, as the extraction time was increased from 6 h up to 8 h. This may be due to degradation of the polysaccharides because of the extended extraction time. The extraction process was performed with temperatures from 50 °C to 100 °C, with the other extraction variables such as ratio of water to raw material and extraction time fixed at 13 ml/g and 3.5 h, respectively. The extraction yield of crude extract increased with increasing extraction temperature and peaked at 7.89 at 100 °C.

Different ratios of water to raw material have considerable effect on extraction yield. In this study, we used ratios of water to raw material from 3 to 30 ml/g, with fixed parameters (extraction time and extraction temperature at 3.5 h and 75 °C, respectively). Extraction yield increased noticeably from 6.10 to 8.10 with ratio of water to material varying from 3 ml/g to 24 ml/g. As extraction time, at the higher experimental levels, as the ratio of water to raw material was increased (from 24 up to 30 ml/g) the yield of crude extract was not changed considerably. Under the appropriate condition of ratio of water to raw material, the crude extract molecules can swell thoroughly, and more compounds could dissolve in water to improve extraction yield.

The determination coefficient (R2 = 0.9897) suggested that the model was valid, implying that 98.97% of the variation could be explained by the fitted model. The adjusted determination coefficient was used to evaluate the correlation between the experimental values and predicted values, and the outcome (R^2_{Adj}= 0.9794) suggested that the correlation was significant. The f-value (1.49) for "the lack of fit" indicated that the "lack of fit" was not significant relative to the pure error ($p > 0.05$). The CV for yield of MN leaves was 0.64%, which defined a good reliability of the experimental values. The adequate precision value 21.61 and PRESS 0.42 for our model indicated an appropriate model could be designed. On multiple regression analysis, the quadratic polynomial equation for the independent variables and response variable expressed as follows:

$$Y = 7.82 + 1.025X_1 + 3.241667X_2 + 2.031X_3 + 0.525X_1X_2 - 0.7X_1X_3 + 0.675X_2X_3 - 0.206X_1^2 - 0.21833X_2^2$$

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Where, Y is extraction yield (%); X_1, extraction time (h); X_2, extraction temperature; and X_3, ratio of water to raw material (ml/g)

Statistical analysis of the results showed that the optimal conditions for higher extraction yield were extraction time: 6 h, extraction temperature: 90°C, and the ratio of water to raw material: 19. Under these conditions, the experimental yield was 9.22% ± 0.47%, which well matched the predicted value (9.18%), under these conditions; the experimental yield was 9.18%.

Furthermore, results obtained from chemical analysis showed that DPPH, OH and super oxide scavenging of MN crude extract increased rapidly with increasing of its concentration. DPPH scavenging rate of the crude extract was more than BHT standard (89% vs. 78%) at their maximum equivalent concentration (300 ppm). Furthermore, OH and super oxide scavenging ability of MN crude extract (87% and 91%, respectively) were significantly more than ascorbic acid standard solutions (74% and 81%, respectively) at their highest experimental concentrations (150 ppm). Based on our results, components extracted from *Malva neglecta* leaves may introduce as an antioxidant and free radical scavenger. This study could help food industries to add a new source of hydrocolloid with certain functionality as an alternative additive in different foods, cosmetic and pharmaceutical products.

Keywords: *Malva neglecta;* Extraction; Crude extract; Antioxidant property; Free radical.